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Sodium and Chloride Transport by the Intestine of the European Flounder *Platichthys flesus* Adapted to Fresh or Sea Water

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Summary. 1. Everted intestinal sacs prepared from *Platichthys flesus* adapted to sea water (SW) have higher rates of salt and water transport than did sacs prepared from fresh water adapted (FW) fish.

2. Intestines mounted in Ussing chambers gave stable open-circuit voltages and short-circuit currents after pre-incubation for 20—30 min of -1.91 ± 0.14 (14) mV and -45.0 ± 5.1 (14) $\mu A/cm^2$, SW fish, and -1.24 ± 0.14 (11) mV and -18.2 ± 3.6 (11) $\mu A/cm^2$, FW fish.

3. Isotope flux measurements indicated a net Na transport of 21.5 ± 4.1 (11) $neq/cm^2 \cdot min$ in SW fish intestines, but no significant Na transport in FW fish (7.6 ± 7.6 (9) $neq/cm^2 \cdot min$). Both preparations showed an active Cl transport, 26.6 ± 6.1 (12) $neq/cm^2 \cdot min$ for SW and 18.5 ± 9.7 (17) $neq/cm^2 \cdot min$ for FW fish.

4. No significant difference in the uptake of Na or Cl across the mucosa was observed between FW and SW fish. The uptake of both ions showed some saturation at high concentrations.

5. Interactions between Na and Cl uptake were small, although Cl uptake was significantly *higher* in Na-free solutions.

6. It is concluded that electrogenic Cl transport may be the dominant mechanism for water and salt transport in flounder intestine, and that adaptation to a saline environment involves regulation of pumping rather than of Na or Cl entry across the mucosal membrane.

Key words: Sodium and Chloride Transport — Intestinal Mucosa Permeability — Fish Osmoregulation.

Marine teleost fishes maintain their osmotic equilibrium by drinking sea water, and absorbing relatively large amounts of salt and water across the intestine; excess salt being subsequently excreted across the gills (Smith, 1930). Certain teleosts such as the eel can adapt to a fresh water environment. When this happens the rate of salt and water absorption across the intestine is considerably reduced [9, 13]. Recent

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evidence suggests [6] that in the intestine of these euryhaline fishes, in contrast with mammalian small intestine, Cl rather than Na transport may be the dominant factor for salt and water absorption. In fact, in intestines from *A. japonica* adapted to either fresh or salt water, the active Na transport remains about constant, whilst the active Cl transport is higher in salt water adapted animals [5]. The flounder taken from sea water, also shows a significant active Cl flux, part of this flux being dependent on Na [6]. The flounder also can adapt to a fresh water environment and it seemed interesting to compare its ability to absorb salt under these conditions with what was known for the eel and if possible to relate this to the mechanism whereby Cl crosses the mammalian intestine *via* a Na-dependent mechnism [8]. The present experiments were therefore undertaken to investigate the transport of Na and Cl in the intestine of the European flounder *Platichthys flesus*, to see which parameter would regulate salt and water transport during adaptation to fresh water. A preliminary report of some of these findings has already been published [7].

Methods

Flounders weighing about 200 g were shipped by air from Nantes to Nice, and maintained without food at 16° for at least 1 week in sea or fresh water before use. Samples of blood, and where possible intestinal fluid, were taken to determine electrolyte concentration (sodium by flame photometry, and chloride by electrometric titration) and total osmotic pressure. The whole intestine from 2 cm posterior of the stomach to 3 cm anterior to the cloaca was dissected out, and a portion about 2 cm in length mounted in a small Perspex Ussing-type chamber for measuring the transepithelial potential, current and isotopic fluxes [3]. A second posterior portion of the intestine was sometimes used to prepare everted sacs for measuring fluid and sodium transport. Fluxes were calculated as neq Na or nl water per g wet weight per hour incubation.

During a preliminary series of experiments in the Ussing chamber the response of potential and short circuit current with time was determined for fish adapted to fresh and salt water (Fig. 1). Since both membrane potential and scc were constant from 40—80 min after mounting the preparation, tracer fluxes were measured over three 10 min intervals during the period 50—80 min, radioactive media being introduced 25 min earlier to allow isotopic equilibration within the tissue. All experiments were carried out at room temperature (21—24° C) in Krebs-Henseleit medium gassed with 95% O_2/5% CO_2, and containing 5 mM glucose (Krebs & Henseleit, 1932). Unidirectional chloride fluxes were measured with $Na^{36}Cl$, 0.2 μc/ml. Bidirectional Na fluxes were measured with the simultaneous addition of 0.2 μc/ml $^{22}NaCl$ to the mucosal and 5 μc $^{24}NaCl$/ml to the serosal medium bathing the tissue.

Sodium and Chloride Uptake. The rapid uptake of sodium and chloride was measured by the technique of Schultz, Curran, Chez & Fuisz [10], modified for fish intestine [4]. The anterior 6 cm of intestine was mounted as a flat sheet in an apparatus containing 8 sampling ports, and equilibrated with gassed circulating Krebs-Henseleit medium for 30 min. Each port in turn was then exposed to a radioactive medium similar in composition to Krebs-Henseleit medium but con

taining $^{24}NaCl$ (10 μc/ml), and/or $Na^{36}Cl$ (0.5 μc/ml) and ^{3}H-inulin (5 μc/ml). After a short time (determined from preliminary experiments as 45 sec, Fig. 2), the port was washed with a large excess (5 ml) of ice cold isotonic mannitol, and the portion excised, digested in 0.1 N HNO_3 and processed for Cerenkov and liquid scintillation counting as previously described [4]. The inulin space was used to correct for extracellular components of sodium and chloride uptake. When media of differing Na and Cl concentrations were required, Na was replaced by choline, and Cl by SO_4, mannitol being added to maintain constant osmolality. Experiments were designed to minimise any effects of variations along the length of the intestine by pairing the first and last samples for the same experimental treatment.

Results

Transflux in Everted Sacs

Everted intestinal sacs from 7 flounders adapted to salt water gave a mean absorption of water and sodium of 166 ± 70 [7] nl/g · hr and 240 ± 100 [7] neq/g · hr. In 6 experiments with everted sacs from freshwater adapted fish, four intestines gave no net transport, whilst the other two gave rates of water transflux of 91 and 31 nl/g · hr, the corresponding Na fluxes being 140 and 141 neq/g · hr. It thus seems clear that, as in the eel, the absorption of sodium and water is reduced in the European flounder on adaptation to fresh water.

Potential Measurements

For intestines mounted in the Ussing chambers under open circuit conditions, the transepithelial PD was always initially positive (serosal side) in tissues from FW fish and negative in intestines from SW fish. However, the potential changed rapidly with time and stabilized after 20—30 min at a mean value of -1.91 ± 0.14 [14] mV for SW fish and -1.24 ± 0.14 [11] mV for FW fish. Under these conditions the measured short circuit current was -45.0 ± 5.1 [14] and -18.2 ± 3.6 [11] μA/cm² respectively (Fig. 1). Huang & Chen [6] obtained values of -3.35 ± 1.46 mV and -26.5 ± 13.3 μA/cm² for the American flounder *(Pseudopleuronectes americanus)* in sea water using a 5.5 mM glucose saline. The values of PD and scc remained steady for at least 1 hr after the initial 20—30 min equilibration period, during which steady period, tracer fluxes were determined.

Isotopic Measurements of Ion Fluxes

The results of 2 series of determinations of isotopic fluxes across FW and SW intestines are given in Table 1. Because the small intestine is a low resistance tissue, with large unidirectional fluxes, net Na and Cl fluxes represent a small component of the total and are therefore subject to large errors. It can be seen (Table 1) that the unidirectional Na

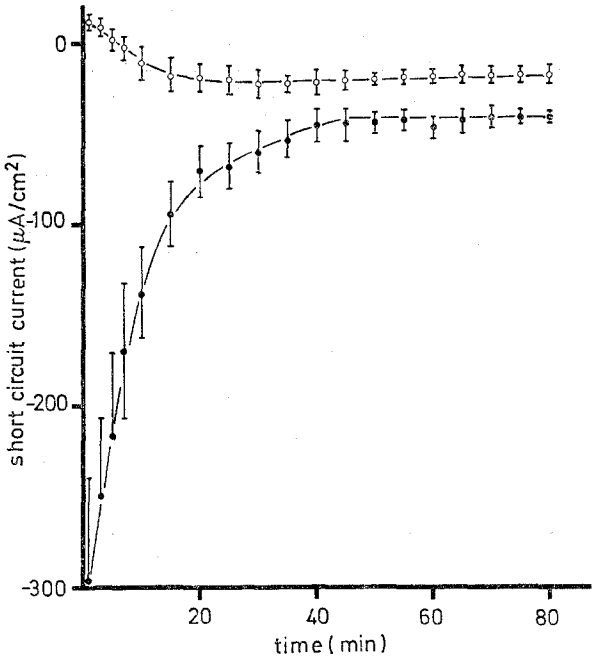

Fig. 1. Short circuit current measurements for isolated intestines for SW and FW flounders. Intestines from FW (○) or SW (●) adapted fishes were mounted in Krebs-Henseleit medium (NaCl 117 mM, KCl 5.7 mM, $MgSO_4$ 1.1 mM, NaH_2PO_4 1.17 mM, $CaCl_2$ 2.54 mM, $NaHCO_3$ 25 mM, glucose 5 mM) at 23° C. Results represent the mean ± S.E. for 14 SW or 11 FW fish

fluxes are larger than Cl fluxes, although the net flux J_{Cl}^{net} may be larger than J_{Na}^{net}. In an attempt to measure J_{Na}^{net} more precisely, bidirectional Na fluxes were determined simultaneously using ^{22}Na and ^{24}Na. In these experiments (Table 1) it can be seen that, whilst there is a significant J_{Na}^{net} of 21.5 ± 4.1 neq/cm² · min in SW fish, the J_{Na}^{net} at 7.6 ± 7.6 neq/cm² · min in FW fishes is greatly reduced and not significantly different from zero. The J_{Cl}^{net} shows an apparent fall from 26.6 ± 6.1 in SW to 15.8 ± 5.9 neq/cm² · min in FW adapted fish, the difference being significant at the 3% level. The transport equivalent of the negative scc in the unidirectional flux experiments is 32 ± 4.5 neq/cm² · min for SW fish and 14.3 ± 4.4 neq/cm² · min in FW adapted fish, neither of which differs significantly from the net Cl flux. One would expect Na transport in SW intestines to generate a short-circuit current if the mechanism were similar to that found in mammals [11] but Na transport in American flounder, measured in Na_2SO_4 Ringer has been shown to be five times greater than the short-circuit current [6]. It is suggested in the present

Table 1. Ion fluxes across intestines from flounders adapted to fresh and salt water

	Fresh water			Salt water		
	Fluxes (neq/cm²/min ± S.E.)					
	J_{sm}	J_{ms}	J_{net}	J_{sm}	J_{ms}	J_{net}
Unidirectional fluxes						
Na	64.7 ± 6.0 (12)	73.8 ± 4.9 (18)	9.1 ± 7.7	46.4 ± 7.6 (17)	64.9 ± 6.1 (29)	18.5 ± 9.7
Cl	21.6 ± 4.2 (12)	37.4 ± 4.2 (21)	15.8 ± 5.9	21.5 ± 2.4 (12)	48.1 ± 5.6 (24)	26.6 ± 6.1
Bidirectional fluxes						
Na	61.1 ± 7.5 (9)	68.7 ± 6.1 (9)	7.6 ± 7.6 (9)	34.2 ± 6.2 (11)	55.7 ± 9.7 (11)	21.5 ± 4.1 (11)

experiments that an active Cl transport accounts for virtually all the net Cl flux, net Na transport being coupled to another anion, possibly HCO_3^-.

Sodium and Chloride Uptake

The results of initial experiments to determine the time course of Na and Cl uptake from Krebs-Henseleit medium are presented in Fig. 2. The data are best fitted by straight lines which do not pass through the origin but give a positive intercept, although the errors on the data do not in fact make this intercept statistically different from zero. The The intercept may represent a space available to Na and Cl but not to inulin, but in any case makes up only a small fraction ($\sim 15\%$) of the uptake at 45 sec, the time chosen for subsequent experiments. In general, uptake experiments with flounder intestine showed much greater variance between samples than previous experiments with *Carassius auratus* [2], perhaps reflecting a real variability in structure.

Fig. 3 illustrates a series of measurements of Na uptake at various external Na concentrations for both SW and FW fish. It is clear that Na uptake tends to saturate with rising Na concentrations, and that there is no apparent difference in the rate of Na uptake between FW and SW adapted fish. This is in contrast with previous results for the goldfish, where there was decreased Na uptake in salt-adapted fish [2]. Since the Na concentration dependence curve showed some saturation, further experiments to investigate differences in Na uptake between SW and FW fish were carried out at 30 mM Na. The mean values obtained, 61.9 ± 4.1 [12] and 64.4 ± 5.9 [12] nmol/cm² · min respectively were not significantly different.

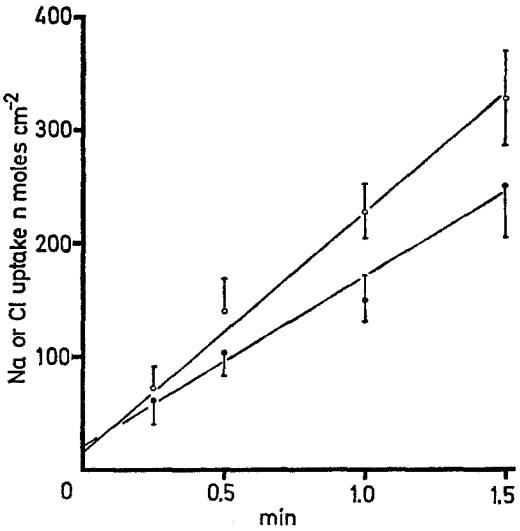

Fig. 2. Time course of the uptake of Na and Cl by flounder intestine. Data presented are the mean ± S.E. of 2 determinations at each time on 4 SW adapted fish. (○) Na, (●) Cl. Medium is modified Krebs-Henseleit to give Na = 140 mM, Cl = 120 mM. Lines drawn by eye

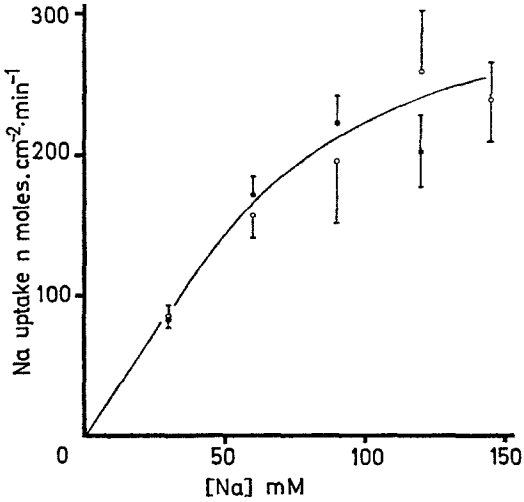

Fig. 3. Concentration dependence of sodium uptake in intestines from FW and SW flounders. (○) SW, (●) FW fish. Data are the mean ± S.E. for 2 determinations at each concentration for 6 SW or FW fish. Na replaced by choline in the modified Krebs-Henseleit medium

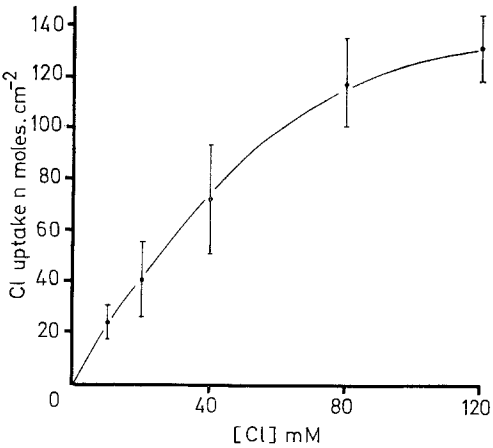

Fig. 4. Concentration dependence of chloride uptake (for 45 sec exposure time) in SW flounder intestine. Data are the mean ± S.E. of 2 determinations for each concentration on 4 SW fish. Chloride replaced by sulphate + mannitol

Chloride uptake from Krebs-Henseleit medium was also measured in both SW and FW fish, the mean values being 176.6 ± 15.4 [12] and 143.7 ± 11.1 [12] nmol/cm² · min respectively. Concentration dependence of Cl uptake was measured in SW fish intestines, the results being represented in Fig. 4. There is again a tendency towards saturation. Initial measurements of Na-dependence of Cl uptake were made by simultaneously determining Cl uptake in the experiment of Fig. 3, i.e. Cl 127 mM, Na varying from 30—120 mM. No significant trend in Cl uptake with varying Na concentration was observed, the uptake being about constant around 130 nmol/cm² as in Fig. 4. However, since Nellans *et al.* [8] have demonstrated a Na-dependent Cl entry step intro mammalian intestine, and Cl transport is probably the dominant mechanism in the flounder, it seemed worthwhile testing this point further. A series of comparisons of Cl uptake in either 0 Na or 120 mM Na, using 30 mM Cl in the medium were therefore made. For intestines from FW fish, the mean Cl uptake in the presence of Na was 33.2 ± 16.1 [12] nmol/cm² · min, whilst in Na-free medium the uptake was 45.2 ± 17 [12] nmol/cm² · min. The large errors represent variations in fishes, the range of uptakes being 11.7—69 nmol/cm² · min. Since the two conditions were tested alternately along the strip of intestine, the data can be treated as paired comparisons. Using this approach the uptake in Na-free media was always higher than in the presence of Na, the mean difference being 12.0 ± 6.5 (48) nmoles/cm² · min. Similar experiments on SW fish suggested a similar trend although the actual results showed larger

errors, the mean difference between paired comparisons being 15.1 ± 11 [28] nmol/cm^2 · min. This surprising finding of a stimulation of Cl uptake in the absence of Na is in direct contrast to the mammalian system and may indicate a specific mechanism for Cl transport in the flounder intestine.

Discussion

The present results show that, following adaptation from SW to FW there is a marked fall in the intestinal transport of salt and water in the European flounder. Similar results have been obtained for the eel, another euryhaline teleost (Oide & Utida, 1964) but the reverse situation has been reported for the goldfish, a stenohaline fish. In the goldfish Na and water transport is much higher in FW fishes than in those living in 200 mM saline [2]. In the FW goldfish intestine there is normally a positive (serosal) P.D. and scc across the intestine, with a net Na transport component equal to the scc. In fishes kept at increasing salinities, the net Na transport and scc falls to about one third of control values. This fall is accompanied by a marked decrease in the permeability of the mucosal membrane to Na. The flounder is in complete contrast to the goldfish; it has a *negative* PD and scc, with the scc equal to a net active Cl transport component. The electrogenic Cl transport dominates this tissue, and the reduction in salt and water transport on transfer from SW to FW is not mediated by a decreased mucosal permeability to Na or Cl. The present data do not allow one to decide unequivocally whether there is a mechanism for active Na transport separate from an active Cl transport system. Certainly on transfer from SW to FW there is a fall in net Na transport, but the errors involved in making unidirectional ^{36}Cl flux measurements on separate preparations make it impossible to decide definitively that the apparent fall in net Cl flux on adaptation from SW to FW is significant. Huang & Chen [6] demonstrated a significant net Na transport in the American flounder after replacing all the Cl in the medium by SO_4. This suggests that at least a third of the active Na transport in this tissue is independent of Cl. Hirano & Utida [5] demonstrated an active Na transport component of about equal magnitude in eels adapted to either FW or SW, but an increased active Cl absorption in SW eels. In therefore seems that there are 2 components of NaCl transport in the euryhaline teleost intestine, a relatively large electrogenic active Cl transport which will be followed by Na moving passively and a smaller, possibly non-electrogenic, transport of Na with some other anion. In the European flounder adaptation from SW to FW probably involves a decrease in both parameters, the Na transport being the easier to demonstrate operationally because of the ability to carry out bidirectional flux measurements.

The observations of an *increased* mucosal Cl uptake in the absence of Na in the bathing medium contrasts with the finding of Nellans *et al.* [8] on the rabbit ileum. It is unlikely that this effect could be caused indirectly, or by a change in potential across the membrane, since it is known that in goldfish intestine replacement of Na by choline causes a hyperpolarisation of the mucosal potential [1]. Since the concentration dependence curve for Cl uptake does show a tendency to saturate at high Cl concentrations, it is quite likely that a component of the uptake is *via* a saturable carrier-type mechanism. Clearly the specificity of any chloride carrier in the flounder intestine must be very different from the Na symporter system in rabbit ileum but more information on its specificity is necessary to define the differences between the two systems.

In conclusion, it seems that regulation of salt and water transport on adaptation to FW in the European flounder *Platichthys flesus* involves a reduction in both a Na and a Cl transporting mechanism. This effect is not achieved by regulating the mucosal permeability (as seems to be the case in *Carassius auratus*) but whether it is effected at the metabolic level by regulating the availability of energy supplies, or directly at the membrane level by blocking pumping activity has yet to be determined.

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